Synthesis of Oxime-Linked Mucin Mimics containing the Tumor-Related T_N and Sialyl T_N Antigens

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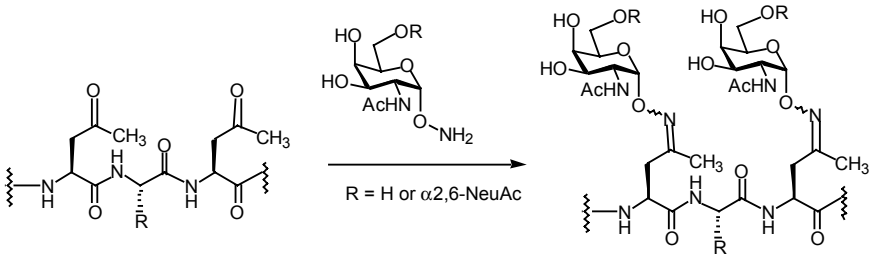
ABSTRACT



The synthesis of oxime-linked mucin mimics was accomplished via the incorporation of multiple ketone residues into a peptide followed by reaction with aminooxy sugars corresponding to the tumor-related T_N and sialyl T_N (ST_N) antigens.

The site-specific attachment of oligosaccharides to proteins can be accomplished by reaction of nucleophilic sugar derivatives with aldehydes and ketones.[1] Previously, we reported on the use of ketone-amino acid **1** (Figure 1) for the synthesis of glycopeptide analogs containing unnatural sugar-peptide linkages.[2] Amino acid **1** can be prepared in one step via reductive ozonolysis of commercially available Fmoc-dehydroleucine (**2**)[3] and can be incorporated into peptides (**3**) by Fmoc-based solid-phase peptide synthesis (SPPS) without need for protection of the ketone group.[4] The ketone group is chemically orthogonal to all naturally occurring amino acid side chain functional groups and thus can be selectively condensed with aminooxy sugars to give the corresponding oxime-linked products (**4**). Since glycoproteins often contain more than one site of glycosylation, we were interested to see if this strategy could be applied to the synthesis of glycopeptides with clustered oxime-linked glycans.

Mucins are a class of heavily *O*-glycosylated proteins that are abundantly secreted by epthelial cells specialized for mucus production.[5] They are rich in serine and threonine residues bearing α-linked glycans initiated by *N*-acetylgalactosamine (GalNAc). A variety of carbohydrate ligands required for cell-surface interactions, including the Lewis and blood group antigens, can be presented on a

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[1] Reviewed in: (a) Marcaurelle, L.A.; Bertozzi, C.R. *Chem. Eur. J.* **1999**, *5*, 1384; (b) Hang, H., Bertozzi; C.R. *Acc. Chem. Res.* **2001**, in press.

[2] (a) Marcaurelle, L.A.; Rodriguez, E.C, Bertozzi, C.R *Tetrahedron Lett.* **1998**, *39*, 8417; (b) Rodriguez, E.C.; Marcaurelle, L.A.; Bertozzi, C.R. *J. Org. Chem.* **1998**, *63*, 9614.

[3] Purchased from BACHEM.

[4] Marcaurelle, L.A.; Bertozzi, C.R. *Tetrahedron Lett.* **1998**, *39*, 7279.

[5] Reviewed in: (a) Hanisch, F.-G. *Biol. Chem.* **2001**, 143; (b) Strous, Dekker, *J. Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 57; (c) Carraway, K.L.; Hull, S.R. *Glycobiology*, **1991**, *1*, 131.

mucin scaffold. In cancer cells, the glycosylation pattern of mucins is altered, leading to the expression of distinct tumor-related epitopes.[6] Thus, mucin fragments and their cancer-associated oligosaccharides have attracted much attention as components of synthetic cancer vaccines.[7] In the present study we focused on the preparation of oxime-linked mucin mimics containing clusters of the T_N and sialyl T_N (ST_N) antigens[8] (Figure 2), which are abundantly expressed in many types of cancer, including tumors of the breast, colon, liver and pancreas.[9]

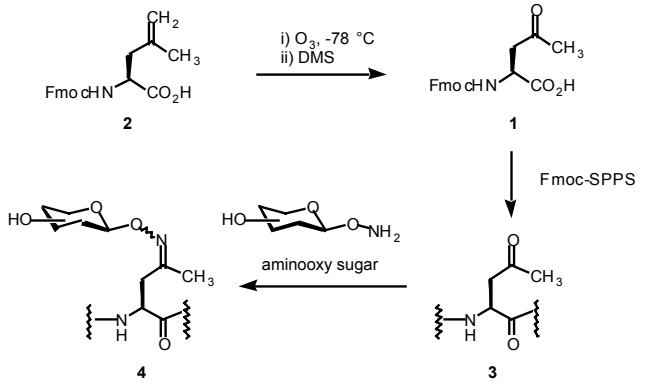


Figure 1. Synthesis of oxime-linked glycopeptides.

We chose fragments of the endothelial mucin GlyCAM-1 (**5** and **6**, Figure 3) as peptide scaffolds.[10] Peptides **5** and **6** each replace six Ser or Thr residues within the 12- or 17-amino acid sequence with amino acid **1** (designated with the single letter code "O"). The syntheses of **5** and **6** were carried out on an automated peptide synthesizer using DCC/HOBt mediated couplings, on MBHA and Fmoc-Glu(tBu)-Wang resins, respectively. Amino acid **1** was used without protection of the ketone group. Following chain assembly the peptides were cleaved from the resin with 95% aqueous TFA for 5 hours and precipitated from Et_2O. Analysis of the crude peptides by ESI-MS showed the desired ketone-containing derivatives as the major products.[11] Purification of the peptides by reversed-phase HPLC yielded targets **5** and **6** in 40-46% yield.

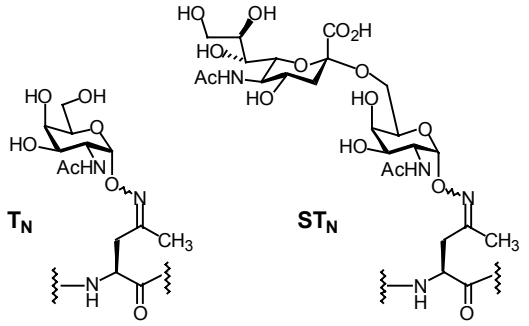


Figure 2. Oxime-linked analogs of the T_N and ST_N antigens.

GlyCAM-1:

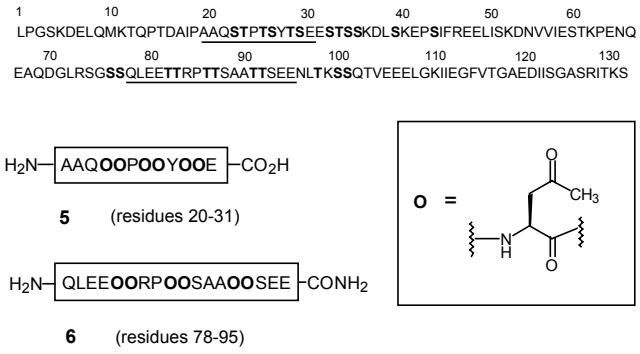


Figure 3. Amino acid sequence of GlyCAM-1 and corresponding peptide fragments **5** and **6** bearing ketone-amino acid **1** (single letter code "O") in place of Ser and Thr.

The synthesis of the aminooxy T_N antigen (**7**) was accomplished as previously described[2] using a phase transfer catalyzed (PTC)[12] reaction of *N*-hydroxysuccinimide (NHS) with glycosyl chloride **8**[13] (Scheme 1). Reductive acetylation of **9**, followed by mild hydrazinolysis afforded the desired aminooxy sugar (**7**). We have recently found that the intermediate NHS

[6] Varki, A. Glycosylation Changes in Cancer. In *Essentials of Glycobiology*; Varki, A.; Cummings, R.; Esko, J., Freeze, H.; Hart, G.; Marth, J.; Eds.; New York: Cold Spring Harbor Laboratory, 1999; pp 537-550.

[7] Danishefsky, S.J; Allen, J.R. *Angew. Chem., Int. Ed.* **2000**, *39,* 836.

[8] For the synthesis of glycopeptides containing the T_N and ST_N antigens see, (a) Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 618; (b) Keil, S.; Claus, C.; Dippold, W.; Kunz, H. *Angew. Chem., Int. Ed.* **2001**, *40*, 366; (c) Kuduk, S.D.; Schwarz, J.B.; Chen, X.-T.; Glunz, P.W.; Sames, D.; Ragupathi, G.; Livingston, P.O.; Danishefsky, S.J. *J. Am. Chem. Soc*. **1998**, *120*, 12474; (d) Schwarz, J.B.; Kuduk, S.D.; Chen, X.-T.; Sames, D.; Glunz, P.W.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1999**, *121*, 2662; (e) Elofsson, M.; Salvador, L.A.; Kilhberg, J. *Tetrahedron* **1997**, *53*, 369; (f) George, S.K.; Holm, B.; Reis, C.; Schwientek, T.; Clausen, H.; Kihlberg, J. *J. Chem. Soc. Perkin Trans.* 1 **2001**, 880.

[9] (a) Brockhausen, I.; Yang, J.M., Burchell, J.; Whitehouse, C.; Taylor-Papdimitriou, J. *Eur. J. Biochem.* **1995**, *233*, 607; (b) Sasaki, M.; Yamato, T.; Nakanuma, Y. *Pathol. Int.* **1999**, 49, 325; (c) Itzkowitz, S.H.; Yaun, M.; Montgomery, C.K.; Kjeldsen, T.; Takahashi, H.K.; Bigbee, W.L.; Kim, Y.S. *Cancer Res.* **1989**, *49*, 197; (d) Itzkowitz, S.; Kjeldsen, T.; Friera, A.; Hakomori, S.-N.; Yang, U.-S.; Kim, Y.S. *Gastroenterology* **1991**, *100*, 1691.

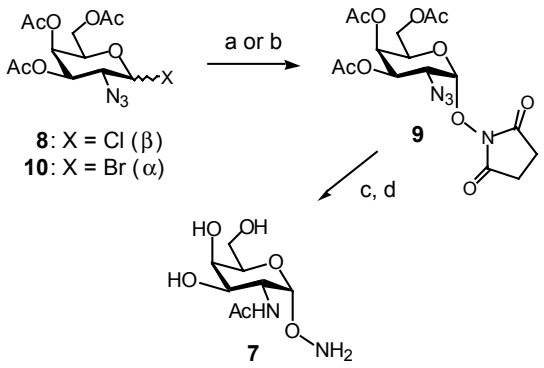
[10] Imai, Y.; Singer, M.S.; Fennie, C.; Lasky, L.A.; Rosen, S.D. *J. Cell. Biol.* **1991**, *113*, 1213.

[11] ESI-MS (neg-ion mode): calcd for **5** 1356.1, found 1355.7; calcd for **6** 2021.8, found 2021.7.

[12] Cao, S.; Tropper, F.D, Roy, R. *Tetrahedron* **1995**, *51*, 6679.

glycoside (**9**) can also be accessed using glycosyl bromide **10**[13] as a donor in a Koenigs-Knorr glycosylation with NHS. While the stereoselectivity of this reaction is not as high as that achieved in the PTC reaction, the preparation of the α-bromide is generally higher yielding than the β-chloride (95% versus 45%)[13] making this route an attractive alternative.

Scheme 1[a]



[a]Reagents: (a) NHS, $(n\text{Bu})_4\text{NHSO}_4$, CH_2Cl_2, Na_2CO_3, 57% (α only); (b) NHS, $AgClO_4$, CH_2Cl_2, 4 Å MS, 67% (3:1 α/β); (c) H_2, Pd/C, Ac_2O, 100%; (d) 10% aq. N_2H_4, 71%.

The synthesis of aminooxy-ST_N **11** utilized the selectively-protected glycosyl acceptor (**12**), containing the pre-installed NHS glycoside, for reaction with known sialyl phosphite **13**[14] (Figure 4). For the installation of the NHS glycoside we chose to use a Koenigs-Knorr glycosylation with glycosyl bromide **14**, which was obtained from 6-*O*-TBDPS-D-galactal (**15**).[15]

As depicted in Scheme 2, glycosyl bromide **14** was generated in three steps via iosopropylidine formation, azidonitration[12] with CAN and NaN_3 and treatment with LiBr. Reaction of bromide **14** with NHS in the presence of $AgClO_4$ gave compound **16** in 65% yield as a mixture of anomers (3:1 α/β). Isolation of the desired α-glycoside (**12**) was achieved following removal of the TBDPS group with TBAF. Glycosylation of **12** with sialyl phosphite **13** using TMSOTf as the promoter gave disaccharide **17** in 44% yield as a mixture of anomers (3:1 α/β). Subsequent deprotection and reductive acetylation of **17** over a series of steps afforded the target aminooxy-ST_N **11**.

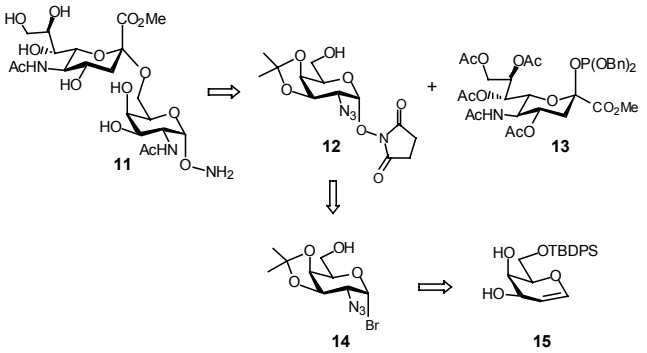
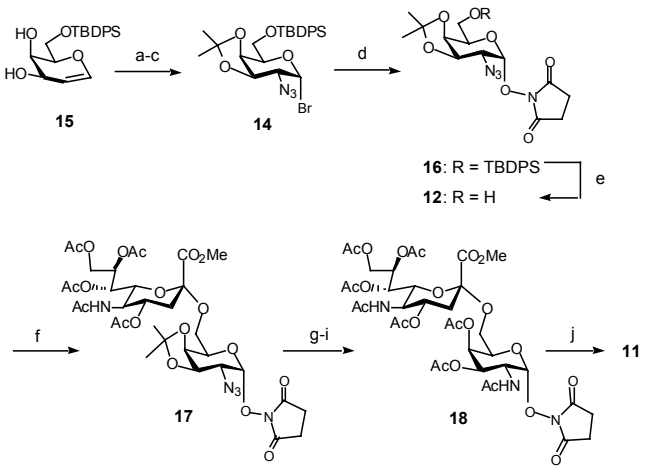


Figure 4. Retrosynthesis of aminooxy-ST_N (**11**).

Scheme 2[a]

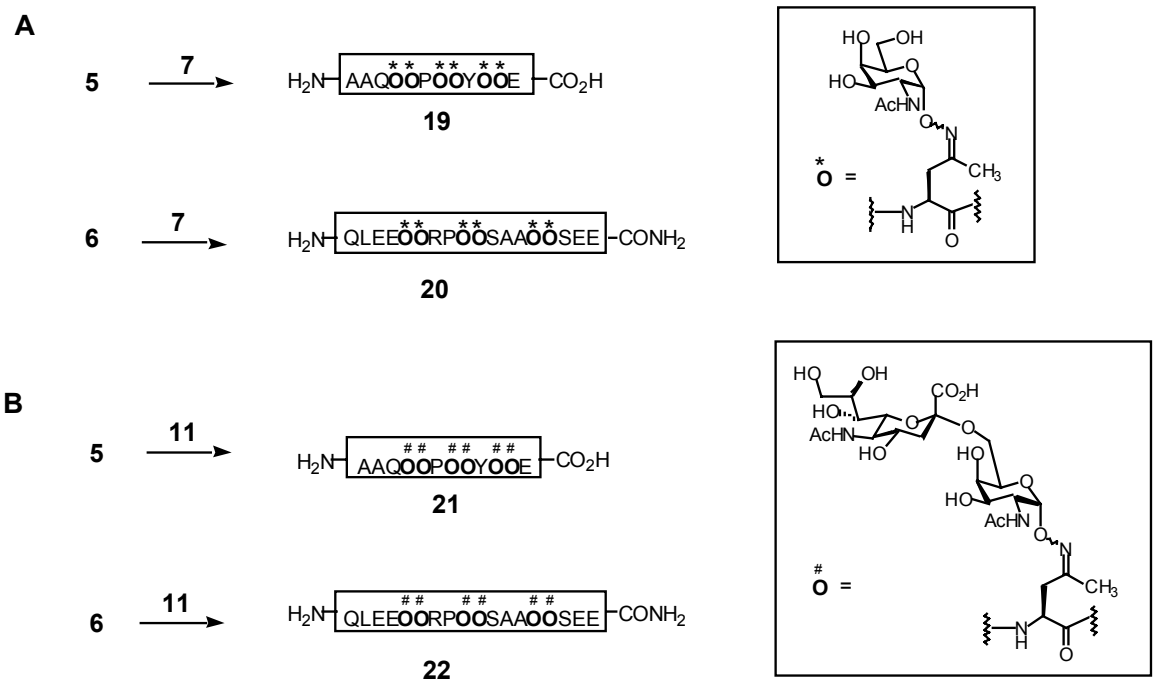


[a]Reagents: (a) $Me_2C(OMe)_2$, PPTS, DMF, 50 °C, 1 h, 95%; (b) CAN, NaN_3, CH_3CN, -20 °C, 15 h, 71%, (c) LiBr, CH_3CN, rt, 5 h, 96%; (d) NHS, $AgClO_4$, CH_2Cl_2, 4 Å MS, rt, 2 d, 65% (3:1 α/β); (e) TBAF, AcOH, THF, rt, 6 h, 45%; (f) **13**, TMSOTf, THF, 4 Å MS, -35 °C, 1 h, 44% (3:1 α/β); (g) *p*-TsOH, MeOH, rt, o.n., 60%; (h) Ac_2O, pyridine, DMAP, rt, o.n., 54%; (i) H_2, Pd/C, Ac_2O, rt, 2 h, 49% after HPLC; (j) i) NaOMe, MeOH, rt, 24 h, ii) LiOH, MeOH, H_2O, 4 °C, o.n., iii) 10% aq. $N_2H_4{\cdot}H_2O$, 70% for 3 steps.

[13] Lemieux, R.; Ratcliffe, R.M. *Can. J. Chem.* **1979**, *57*, 1244.

[14] (a) Sim, M.M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260; (b) Bhattacharya, S.K.; Danishefsky, S.J. *J. Org. Chem.* **2000**, *65*, 144.

[15] Gervay, J.; Peterson, J.M.; Oriyama, T.; Danishefsky, S.J. *J. Org. Chem.* **1993**, *58*, 5465.



Scheme 3. Synthesis of mucin mimics **19** and **20,** containing the T_N antigen (A) and mucin mimics **21** and **22**, containing the ST_N antigen (B). All ligation reactions were performed by incubating the peptide with an excess of aminooxy sugar at 37 °C in NaOAc buffer, pH 5.5.

The ligation of **7** and **11** with peptides **5** and **6** was carried out at 37 °C with an excess of either sugar in NaOAc buffer, pH 5.5 (Scheme 3). The reactions were monitored by reversed-phase HPLC and judged to be complete after 24 h. The oxime-linked products (**19**-**22**) containing the T_N and ST_N antigens were purified by reversed-phase HPLC (60-70% yield) and their identity confirmed by ESI-MS.[16]

These syntheses illustrate that multiple clustered ketone residues can be incorporated into a peptide and reacted with aminooxy sugars. Such an approach circumvents the need to synthesize large quantities of complex glycosyl amino acids for use in peptide synthesis, a process which can be extremely labor intensive depending on the complexity of the pendant glycan. The oxime-based strategy benefits from convergent assembly of peptides and aminooxy sugars, both of which are straightforward to prepare. The incorporation of these glycopeptides into larger, full-length proteins, by techniques such as native and expressed protein ligation,[17] should provide access to homogenous mucin-analogs for a variety of applications.

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Supporting Information Available: Full experimental procedures and tabulated ^1H and ^{13}C NMR data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

[16] ESI-MS (neg-ion mode): calcd for **19** 2665.3, found 2665.6; calcd for **20** 3331.3, found 3330.2; calcd for **21** 4412.9, found 4413.2; calcd. for **22** 5078.9, found 5080.0.

[17] (a) MacMillan, D.; Bertozzi, C.R. *Tetrahedron* **2000**, *56*, 9515; (b) Tilbert, T.J.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122,* 5421; (c) Marcaurelle, L.A.; Bertozzi, C.R. *Chem Eur. J.* **2000**, *7*, 1129; (d) Dawson, P.E.; Kent, S.B.H. *Ann. Rev. Biochem.* **2000**, *69*, 923; (e) Muir, T.W.; Sondi, D.; Cole, P.A. *Proc. Natl. Acad. Sci*. **1998**, *95*, 6705.